EXTRA VIRGIN OLIVE OIL: PROCESSING, QUALITY, SAFETY, AUTHENTICITY, NUTRITIONAL AND HEALTH ASPECTS

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Contents

1. Introduction

Olive is one of the most ancient fruit tree species that is cultivated in the Mediterranean Basin, with great socioeconomic impact for the countries present in the area. Olive production throughout the world uses more than 1275 cultivars and 4200 genotypes (Bartolini et al., 2005). Most cultivars have been identified in southern European countries, including 538 in Italy, 183 in Spain, 88 in France and 52 in Greece (Ipec et al., 2009). However, Greece has more cultivars than those described in the National Catalogue of Agricultural Plant Varieties, the majority of which remain unexploited.

Many studies have acknowledged olive oil as a healthy food with multiple utilities in, and benefits for, the human body (Preddy and Watson, 2010; Galaris et al., 2010). The consumption of extra virgin olive oil (EVOO) is steadily increasing due to its unique sensory, nutritive qualities, biological properties and health promoting effects. Greece is the third olive oil producing country in the world after Spain and Italy, with about 16% of the global annual production of which 80% being extra virgin. Hence, the superior quality of Greek olive oil, the significant diversity, the domestic diversification and its recognized nutritional value are tangible evidence of the importance that should be given for the promotion of Greek olive oil as a precious national product. However, the Greek market has been unable until now to properly and fully exploit it.

Geographically speaking, almost 70% of olive oil production in Greece is centered in two regions—Peloponnese (39%) and Crete (30%)—with the prefecture of Messinia being the dominant olive-growing area of Peloponnese. Koroneiki cultivar (*Olea europeae* var. *Microcarpa alba*) is the indigenous variety in Messinia—the name of which derives from Koroni, a small seaside village southeast of Messinia.

Although there are many research publications related to Koroneiki cultivar in different areas in Greece, no systematic work has been carried out on olive oil analysis from the Messinia region except that published recently by the group of Skiada et al. (2019). In August 2015, the European Commission approved the extension of the "Kalamata Protected Designation of Origin (PDO) olive oil" from the former province of Kalamata to the rest Regional Unit of Messinia, considerably enlarging the area covered by the PDO.

The Mediterranean diet, in which olive oil is the main source of fat has been associated with a decrease in overall, cardiovascular, and cancer mortality. The beneficial effects of olive oil on Coronary Heart Disease (CHD) risk factors are now recognized but often only attributed to the high MonoUnsaturated Fatty Acid (MUFA) content of the olive oil.

Olive oil, however, is a functional food which besides having a high MUFA level, the oleic acid, contains multiple minor components with biological properties. These components will be analysed.

There is a consensus that polyphenols (main natural antioxidants) are one of the components responsible for these health benefits.

The effects of polyphenols on physiopathological mechanisms of cardiovascular disease have merited attention.

Among these effects are inhibition of platelet aggregation, inhibition of oxidation of Low-Density Lipoproteins (LDLs), stimulation of Nitric Oxide (NO) production and downregulation of the expression of endothelial adhesion molecules.

Polyphenols in EVOO are believed to underlie many of the cardiometabolic benefits of olive oil consumption, among them, lower rates of CardioVascular Disease (CVD) and diabetes, reduced low-density lipoprotein cholesterol and increased High-Density Lipoprotein (HDL) cholesterol, lower blood pressure, improved vascular reactivity, reduced inflammation, and enhanced HDL functionality.

The polyphenol present at highest concentrations in VOO is hydroxytyrosol (3,4-dihydroxyphenylethanol) (HT), if considered the sum of HT as a simple phenol and the HT derived from oleuropein aglycone hydrolysis; its concentration is known to vary depending upon the variety of olive, ripeness, method of oil pressing, and geographical region, among other factors.

The content of the minor components of an olive oil varies, depending on the cultivar, climate, ripeness of the olives at harvesting, and the processing system employed to produce the types of olive oil currently present on the market: virgin, ordinary, or pomace.

Virgin olive oil is produced by direct pressing or centrifugation of the olives. Virgin olive oils with acidity level greater than or equal to 3.3 degrees (International Olive Oil Council Regulation/T.15/NC.n3.Rev2. Nov 24, 2006), or 2 degrees in Europe (European Regulation N. 1513/0) are submitted to a refining process in which some components, mainly phenolic compounds, and to a lesser degree squalene, are lost. By mixing virgin and refined olive oil an ordinary olive oil (olive oil, UE 1991) is produced and marketed. After virgin olive oil production the rest of the olive drupe and seed is again processed, submitted to a refining process, and the resulting pomace olive oil, to which a certain quantity of virgin olive oil is added, is put on the market.

On the other hand, increased globalization of trade and higher cost of olive oil production compared to other vegetable oil sources has led to adulteration with cheaper oils of lower grade. Consequently, a controlled traceability system has become a requirement in the olive oil supply in order to protect consumers against any unapproved and fraudulent practices. Thus, olive oil authenticity and traceability are crucial in order to overcome frauds in the international olive oil trade. For this reason, the European Union has adopted a series of regulations in order to certify, protect, and guarantee the quality of the monovarietal olive oils. The quality of these monovarietal olive oils is associated with specific characteristics directly related to the olive cultivar. Therefore, the authenticity efforts are concentrated on the identification of their botanical origin as well as their adulteration with lower quality or less costly cultivars of lower commercial value.

The scope of this chapter is to review key aspects of EVOO: processing, quality, safety, authenticity, nutritional and health aspects.

2. Processing Aspects

2.1. Two-phase and 3-phase Olive Mills

In the 1970s and 1980s, olive processing by the continuous centrifugation system, called at three-phases, spread over many countries of the Mediterranean area.

This system is called at three-phases because the centrifugal decanter allows the separation of three flows of matter, as olive oil, pomace and vegetable waste water, and it needs lukewarm water added to dilute olive paste, as was shown in some papers. This causes the reduction of natural antioxidants of oil, the production of moister pomace and a considerable volume of vegetable waste water (80–100 l/100 kg of olives.

One possibility to lessen these drawbacks, which has been investigated, is to recycle the vegetable waste water as soon as it is produced and to use it instead of ordinary water to dilute the olive paste which enters the decanter. The results obtained by applying this technique show a 35–40% reduction in the volume of vegetable waste water and an increase of about 30% in the total phenols content of the oil.

At the beginning of the 1990s, some olive oil plant manufacturers have launched new models of decanters in the market. These are able to separate the oily phase from the olive paste without requiring the addition of lukewarm water and without producing vegetable waste water.

These decanters, in fact, have two exits producing oil and pomace, and for this are called at two-phases. They are widespread in Spain and produce a very wet pomace, with a water content variable between 65 and 70% by weight.

3. Quality and Safety Aspects

3.1. Quality-Polyphenols and Other Compounds

Some of the EVOO polyphenols are unique, both because they are exclusively present in this food and for their sensory properties, since they have a very distinctive bitter and pungent taste, among them oleacein and oleocanthal, 2 secoiridoids that are not present originally in olives, but are naturally formed during the production of EVOO. Together with the hydroxytyrosol found in urine after the consumption of EVOO, these molecules are gaining attention for their anti-inflammatory properties. Other interesting bioactive compounds are oleuropein, another polyphenol, and some triterpenes, such as squalene and oleanolic and maslinic acids. In fact, ongoing feeding trials are evaluating the effects of functional olive oils (EVOO enriched with these compounds) to increase the antioxidant and anti-inflammatory effects of the original EVOO.

Part of the salutary health effects of olive oil are due to its content in oleic acid, which is found in all types of olive oil, but an important part is attributable to the >200 minor components that include mainly phenolic compounds, but also tocopherols, phytosterols, carotenoids, luteolin, and triterpenic acids, which are enriched in EVOO.

Among the triglycerides, the major ones are triolein (43.5%), 1-palmityl-2,3-diolein (18.4%) and 1-linoleyl-2,3-diolein (6.8%). The unsaponifiable fraction of VOO, which represents 1–2% of the oil, is made up of different minor compounds. Hydrocarbons may be constituted up to 0.7%, mainly squalene, and low quantities of epoxy-squalene isomers and alkanes (C16-C35). Phytosterols make up the main part of the unsaponifiable fraction of olive oil: b-sitosterol is the most abundant, followed by D5-avenasterol, and then by campesterol and stigmasterol.

Of the tocopherols, a-tocopherol comprises about 90% of the total tocopherol fraction. The major phenolic compounds identified and quantified in olive oil belong to three different classes: simple phenols (hydroxytyrosol, tyrosol), secoiridoids, and lignans. Other constituents of the unsaponifiable matter are carotenoids (β-carotene being the most important), chlorophylls, and pheophytins. The alcohol fraction of VOO include aliphatic alcohols, mainly docosanol, tetracosanol, hexacosanol, and octacosanol, and at trace levels, tricosanol, pentacosanol, and heptacosanol. In smaller quantities, triterpenic alcohols (cycloartenol, 24-methylen-cycloartenol, and a- and b-amirines), diterpenic alcohols (fitol and geranylgeraniol), and triterpenic dialcohols (erythrodiol and uvaol) are also present.

The composition of the unsaponifiable fraction of VOO is affected by several factors such as olive cultivar, altitude, climatology, agronomical factors, time of harvest, olive storage after harvest, and oil extraction system.

3.2. Safety Aspects

3.2.1. The HACCP and ISO22000 Approach

Hazard Analysis Critical Control Points (HACCP) is a structured approach to the identification, assessment of risk (likelihood of occurrence and severity), and control of hazards associated with a food production process or practice.

Design and implementation of a HACCP system involves the well-known seven basic principles or steps including hazard analysis, identification of the Critical Control Points (CCPs) in food preparation, establishment of critical limits for preventive measures associated with each CCP, establishment of procedures to monitor CCP's, establishment of corrective action to be taken when monitoring shows that a critical limit has been exceeded, establishment of an effective record keeping system that documents the HACCP, and establishment of procedures to verify that the HACCP system is working.

ISO 22000 specifies the requirements of a Food Safety Management System, encompassing all the range of food organizations involved in the food chain from farmers to catering businesses. ISO 22000 creates a uniform and homogeneous platform of requirements, acceptable to all authorities worldwide. The adoption of ISO 22000 was carried out in the year 2005 and a new version has been adopted in 2018. These food organizations involve the following categories:

- The directly involved organizations with the food chain, i.e., primary production, food additives manufacturers, raw and auxiliary raw materials for the food industries, food manufacturers, food services, food distributors, pest control companies as well as distribution and warehousing companies.
- The indirectly involved such as suppliers of raw materials, equipment, cleaning and disinfectant solutions, packaging materials and other materials that come directly or indirectly into contact with food.

Table 1 shows for the schematic of CCP and Operational Prerequisite Programs (OPRP) detection in EVOO processing with examples of 2 processing steps according to Decision of EC 2016/C278/01. Table 2 shows the recognition and categorization of hazards (physical, chemical, microbiological). Table 3 (a) depicts a HACCP plan for filtration of olive oil (CCP 1, P) whereas Table 3(b) shows an OPRP plan for storage of packaged olive oil. Figure 1 shows the flow diagram of EVOO Processing.

Processing step	**Receiving of olive fruits**	**Filtration**
Q1. Do preventative control measures exist? (Yes / No)	**Y**	**Y**
Q2. Is the step specifically designed to eliminate or reduce the likely occurrence of hazard to an acceptable level? (Yes / No)	**N**	**Y**
Q3. Could contamination with identified hazard(s) occur or could this increase to unacceptable levels? (Yes / No)	**Y**	
Q4. Will a subsequent step eliminate identified hazard(s) or reduce likely occurrence to acceptable levels? (Yes / No)	**N**	
Is this step a critical control point? (Yes / No)	**OPRP**	**CCP1 (P)**

Table 1. Tree diagram for CCP and OPRP detection in extra virgin olive oil processing with examples of 2 processing steps (Decision of EC 2016/C278/01)

No.	**Processing steps**	**Hazard**	**Control measures**
1	Receiving and storage of olive fruits	➢ **M:** Presence of pathogenic microorganisms due to inappropriate conditions of receiving and distribution and possible injuries ➢ **C :** Toxic residues in olive fruit, pesticide residues ➢ **P :** Foreign matter, stones etc.	➢ List of approved suppliers ➢ QC plan during receiving ➢ Macroscopic control ➢ Good aeration of olive fruits during transportation ➢ Temperature control of olive fruits ➢ Cleaning and sanitation program of equipment ➢ GMPs, GHPs
2	Destoning and removal of leaves and foreign matter	➢ **P :** Foreign matter, leaves	➢ Macroscopic control for foreign matter ➢ GMP ➢ Equipment maintenance

No.	Processing steps	Hazard	Control measures
3	Washing of olive fruits	➢ **M:** Contamination with pathogenic microorganisms due to contaminated equipment and water ➢ **C :** Contamination with chemical substances from contaminated water ➢ **P:** Foreign matter	➢ Cleaning and sanitation program of equipment ➢ GMPs, GHPs ➢ Microbiological control of water ➢ Chemical control of water ➢ Equipment maintenance
4	Crushing	➢ **P:** Foreign matter ➢ **C:** Contamination from lubricant residues in equipment	➢ Macroscopic control ➢ Equipment maintenance ➢ Use of appropriate food-grade lubricants
5	Malaxation	➢ **M:** Pathogenic microorganisms due to increased temperature and time ➢ **C:** Contamination from lubricant residues in equipment ➢ **P:** Foreign matter	➢ Control of temperature and time. ➢ Equipment maintenance ➢ Use of appropriate food-grade lubricants
6	Centrifugation	➢ **P:** Foreign matter (metals, insects)	➢ Equipment maintenance ➢ Macroscopic control ➢ GMP
7 **CCP 1 (P)**	Filtration	➢ **P:** Foreign matter (metals, plastics, stones) from ineffective filtration	➢ Preventative maintenance ➢ Macroscopic control of filter ➢ GMP
8	Packaging	➢ **P:** Foreign matter (metals) ➢ **C:** Increased rancidity or peroxides in olive oil, contamination from lubricants ➢ **M:** Contamination from pathogenic microorganisms due to contaminated equipment	➢ Macroscopic control ➢ GMPs, GHPs ➢ Equipment maintenance ➢ Temperature control of oil ➢ Good sealing of package to avoid contact of oil with oxygen ➢ Packaging not to be made of ferrous,

No.	Processing steps	Hazard	Control measures
			copper or nickel ➢ Use appropriate food-grade lubricants ➢ Cleaning and disinfection of equipment ➢ CIP
9	Storage	➢ **C:** Increased rancidity or peroxides in olive oil ➢ **M:** Contamination with pathogenic microorganisms e.g. *Salmonella* sp. due to contact of container with dust and dirt, due to bad personnel hygiene, and from pests ➢ **P:** Foreign matter	➢ Storage temperature 10 – 15 °C ➢ Avoidance of intense light ➢ Pest and insect control ➢ FIFO system ➢ Avoidance of open cases ➢ GHP ➢ Cleaning and disinfection programs ➢ GMPs
10	Receiving of packaging materials	➢ **M :** Presence of pathogenic microorganisms ➢ **C:** Migration of chemical residues due to inappropriate packaging materials ➢ **P :** Foreign matter	➢ List of approved suppliers ➢ Agreed specifications ➢ Traceability ➢ Certificates of raw materials ➢ Control of transportation/receiving conditions ➢ Labelling of primary packaging during receiving
11	Storage of packaging materials	➢ **M:** Contamination with pathogenic microorganisms due to contact of packaging materials with dirt, dust, lack of GHP, contamination with pathogenic microorganisms from insects, pests ➢ **P:** Foreign matter	➢ Pest and insect control ➢ Cleaning and disinfection programs ➢ GMPs, GHPs ➢ Personnel training

Table 2. Recognition and Categorization of Hazards (Physical, Chemical, Microbiological)

Processing step	Hazard	Control measures	Critical limits	Monitoring	Corrective action
Filtration	**P:** Foreign matter (metals, plastics, stones) from ineffective filtration	➢ Preventative maintenance ➢ Macroscipic control of filter ➢ GMPs	➢ Good conditions of filters (filter diameter 1 mm) ➢ Absence of foreign matter	Macroscopic control Once per day	➢ In case of a problem in filters lot is quarantined and evaluated ➢ Damaged filters are replaced

(a) HACCP plan for filtration of olive oil (CCP 1, P)

Processing step	Hazard	Control measures	Criteria	Monitoring	Corrective action
Storage	➢ **C:** Increased rancidity or peroxides in olive oil ➢ **M:** Contamination with pathogenic microorganisms e.g. *Salmonella* sp. due to contact of container with dust and dirt, due to bad personnel hygiene, and from pests / ➢ **P :** Presence of foreign matter	➢ Storage temperature 10 – 15 °C, ➢ Avoidance of intense light ➢ Pest/insect control ➢ FIFO system ➢ GHP ➢ Cleaning/disinfection program ➢ GMPs	➢ Room temperature: 10 – 15 °C, ➢ Product expiry date	➢ Temperature monitoring ➢ Control of expiry date ➢ Macroscopic control of storage warehouses ➢ Monitoring of cleaning program ➢ Monitoring of pest and insect control program	If T > 15 ºC correct/adjust Quarantine of products that their shelf life has expired

(b) OPrP Plan for Storage of Packaged Olive Oil

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Table 3. Plans for processing of olive oil (a) HACCP plan for filtration of olive oil (CCP 1, P)
(b) OPrP Plan for Storage of Packaged Olive Oil

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Biographical Sketches

Theodoros Varzakas has a Bachelor (Honours) in Microbiology and Biochemistry (1992), a Ph.D. in Food Science and Technology (1998) and an MBA in Food and Agricultural Management from Reading University, UK (1998). He has also worked as a postdoctoral research staff at the same university. He has worked in large pharmaceutical and multinational food companies in Greece for 5 years and has also got at least 20 years experience in the public sector. Since 2005 he served as Assistant, Associate and Full Professor at the Department of Food Science and Technology, University of Peloponnese, ex Technological Educational Institute of Peloponnese, Greece specializing in issues of food technology, food processing/engineering, food quality and safety. Ex Editor of Cuurent Research in Nutrition and Food Science and now Section Editor in Journal Foods section Food Security and Sustainability. Reviewer in many international journals such as *(International Journal of Food Science & Technology, Journal of Food Engineering, Waste Management, Critical Reviews in Food Science and Nutrition, Italian Journal of Food Science, Journal of Food Processing and Preservation, Journal of Culinary Science and Technology, Journal of Agricultural and Food Chemistry, Journal of Food Quality, Foods, Microorganisms*). He has authored more than 180 research papers and written reviews and has presented more than 160 papers and posters at national and international conferences. He has written and edited four books in Greek, and six in English on sweeteners, biosensors, food engineering, food processing, published by CRC. Participation in many European and national research programs as coordinator or scientific member.

Dr Antoniadou Maria has received her diploma in Dentistry from the Dental School, National and Kapodistrian University of Athens, Greece. While in the graduate program, she was a visiting student at the University of Leeds, UK and an Erasmus student at the dental school of ACTA, Amsterdam, the Netherlands. She studied for her master's degree in dental Biomaterials and was simultaneously specialized in Aesthetic Dentistry at the Department of Operative Dentistry at the University of Athens. Following her master's degree, she worked as a research and clinical associate at the Dental School of Alberts-Ludwigs University in Freiburg, Germany. Later, she obtained her PhD degree in the University of Athens, Greece with a theme concerning the *in vitro* characteristics of polymeric materials. She also holds a master's degree in medical and dental translation and interpretation for the English/Greek languages while she also speaks good French and Spanish. Since 2010, she is an assistant professor at the Department of Operative Dentistry, Dental School of Athens, where she holds economical and administrative position. Furthermore, she was production supervisor of the scientific journal "*Hellenic Stomatological Review*". Apart from the administrative and educational activities she has significant publications in Greek and international scientific journals (currently 60 articles) and books (10). In general, Dr Antoniadou has a demonstrated track record of working in the higher education industry and she is skilled in Budgeting, Wellness Coaching, Oral Health Coaching, Medical Psychology and Patient communication. She is finally a Human Resources Expert with an ICF and AC Accredited certifications, focused in Oral health Coaching and Coaching Research Techniques.